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The primary objectives of this project are to: (a) assess the roles of hepatic and pulmonary presystemic elimination in reducing the bioavailability of low levels of volatile organic chemicals (VOCs) found in drinking water supplies; (b) investigate gastrointestinal (GI) absorption pathways for VOCs; (c) characterize the influence of oil dosage vehicles on the absorption, pharmacokinetics (PK) and toxicity of VOCs, with emphasis on potential mechanisms by which corn oil acts. Substantial progress has been made during the first 2 years of the grant towards achieving each of these objectives. Studies in unanesthetized, freely-moving rats, contrasting the PK of equal doses of VOCs given orally as a single bolus and by constant intragastric (ig) infusion for up to 6 hours, revealed significantly lower peak blood levels and bioavailability in the ig groups. Blood concentrations of well metabolized VOCs, such as trichloroethylene (TCE) and 1,1-dichloroethylene, were so low that they were hardly detectable at low dosage levels in the ig animals. These findings suggest that the liver and lungs may be able to remove virtually all of the trace amounts of VOCs that are usually found in drinking water. (continued on reverse side)

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As anticipated, the toxicity of a potent hepatotoxin, carbon tetrachloride (CCl_4), was considerably lower when the chemical was given to rats by gastric infusion than when given as an oral bolus. PK studies are underway to determine the relative contributions of the GI tract, liver and lungs to presystemic elimination of TCE. The role of the lymphatics in systemic absorption of ingested VOCs has been studied. A surgical technique was developed which allowed collection of lymph from the thoracic duct of unanesthetized rats. Experiments indicated that CCl_4 was absorbed to a limited degree via the lymphatics when given orally in an aqueous emulsion, but that lymphatic absorption was much greater when the chemical was administered in corn oil. Other studies in rats revealed that corn oil markedly delayed and prolonged the GI absorption of CCl_4, thereby diminishing its acute hepatotoxicity. A systems analysis approach was successfully utilized to model the oral absorption of CCl_4 when given in a corn oil vehicle. Subacute studies revealed that CCl_4 was somewhat more hepatotoxic when given for up to 4 weeks in corn oil, although there was little difference from the aqueous vehicle group after 13 weeks of exposure. Additional studies of potential mechanisms by which corn oil alters the PK and toxicity of CCl_4 have shown that corn oil dosing caused increased deposition of triglycerides in the liver, which in turn resulted in deposition of increased levels of CCl_4 in the liver. The high lipid intake did not result in increased lipoperoxidation, nor in an increase in hepatic microsomal cytochrome P-450 levels, nor in altered mixed-function oxidase (drug metabolism) activity. Dietary fatty acids were found to be necessary for optimal activity and induction of P-450 in rat liver. Experiments revealed that dietary fat deprivation did protect against CCl_4 hepatotoxicity, apparently by reducing levels of P-450 isozymes necessary for metabolic activation of CCl_4 to cytotoxic metabolites.

BIOAVAILABILITY OF VOLATILE ORGANICS AND OTHER HYDROCARBONS

FROM ENVIRONMENTAL MEDIA: INGESTION IN DRINKING WATER

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I. OVERALL OBJECTIVE AND SPECIFIC AIMS

The overall objective of the project is to critically evaluate and characterize the bioavailability of low levels of volatile organic chemicals (VOCs) present as contaminants of drinking water supplies in the United States.

Specific aims are to:

(1) Characterize and ascertain the significance of hepatic (i.e., liver) and pulmonary (i.e., lung) presystemic elimination of ingested VOCs. Studies are being conducted to determine how effective presystemic elimination is in removing the small (trace) amounts of VOCs typically consumed in water.

(2) Investigate gastrointestinal (GI) absorption pathways. Studies are being conducted to determine whether short-chain aliphatic halocarbons absorbed from the GI tract enter the portal venous circulation, or bypass the liver via the lymphatic system.

(3) Characterize the influence of oil dosage vehicles/diluents on the absorption, pharmacokinetics (PK) and toxicity of VOCs, as well as investigate potential mechanisms by which corn oil affects the pharmacokinetics and toxicity of orally administered VOCs.

II. PROFESSIONAL PARTICIPANTS ON PROJECT

James V. Bruckner, Ph.D., Principal Investigator

Randall O. Manning, Ph.D., Investigator

James M. Gallo, Ph.D., Investigator

Cham E. Dallas, Ph.D., Co-Investigator

S. Muralidhara, M.S., Research Coordinator

III. STATUS REPORT ON ACCOMPLISHMENTS AND PROGRESS MADE TOWARDS ACHIEVING RESEARCH OBJECTIVES AND SPECIFIC AIMS

A. Determination of the Influence of Dosage Regimen and Exposure Route on the Pharmacokinetics and Toxicity of VOCs

The applicability of oral bolus studies to the assessment of health effects of drinking water contaminants has received relatively little attention. Although VOCs and other organic chemicals are usually given by gavage (orally) as a single bolus, human exposures typically occur on a repetitive, or continuing basis as a person consumes water over the course of the day. As a single oral bolus doses of VOCs should produce relatively high blood levels, it appears likely that bolus doses can cause toxicity by exceeding the capacities of presystemic elimination and of defense systems in target tissues. In contrast, the relatively small quantities of these chemicals absorbed in divided doses should be extensively metabolized and/or exhaled, such that toxic levels are not

reached in tissues. If this is true, toxic effects of VOCs in most target organs should be significantly less pronounced under actual environmental exposure conditions than in experiments in which the chemicals are given as an oral bolus.

Inhalation of VOCs should result in higher levels of VOCs in arterial blood and extrahepatic organs (and therefore greater toxicity) than equivalent oral exposures. Chemicals absorbed in the lungs directly enter the arterial circulation, whereas chemicals absorbed from the GI tract into the venous blood must first pass through the liver and lungs before reaching the systemic circulation. Thus, the liver may effectively remove VOCs from the venous blood, but therefore be at greater risk of toxic injury (upon oral exposure). The liver should be most effective at removing relatively small amounts of VOCs absorbed into the blood following low doses (i.e., at doses that do not exceed the metabolic capacity of the liver).

We have conducted a series of experiments in which the influence of pattern and route of exposure on both PK and target organ toxicity was assessed. Male Sprague-Dawley (S.D.) rats inhaled 100 or 1,000 ppm carbon tetrachloride (CCl_4) for 2 hours through a one-way breathing valve. The administered dose was estimated using the following equation: 0.5 X minute volume X time X inhaled concentration. The factor of 0.5 was utilized as it was assumed, based on the known valvular, mask and anatomical dead space, that 50% of the inhaled CCl_4 actually participated in systemic uptake. The equivalent doses to administer orally were thus calculated to be 18.9 and 186 mg/kg, for the 100 and 1,000 ppm exposures, respectively. The oral doses of CCl_4 were given to rats either as a single bolus or via a surgically-implanted gastric cannula as an aqueous Emulphor® emulsion over 2 hours by constant intragastric infusion (i.g.). Serial blood samples were taken from the subjects and analyzed for CCl_4, in order to obtain blood concentration versus time profiles. Blood and liver specimens were taken 24 hours post exposure for measurement of serum enzyme levels and hepatic microsomal enzymes, which were used as indices of liver injury.

In the foregoing experiments, C_{max}, AUC and hepatotoxicity indices were substantially higher for the oral bolus than the corresponding ig (and inhalation) groups. These results demonstrate that the oral dosage regimen, or pattern of exposure can have a significant impact on toxicity test results. Furthermore, the findings are evidence that toxicity testing protocols should approximate actual exposure conditions as closely as possible. Inhalation resulted in significantly greater C_{max} and AUC values than did ig infusion at both dosage-levels. These data indicate that the liver and lungs are quite efficient in removing CCl_4 from the portal blood, following its absorption from the GI tract (i.e., effective in presystemic elimination). The liver would thus appear to be a greater risk of injury, upon oral exposure, due to greater uptake of CCl_4. Our hepatotoxicity data, however, were not conclusive. There was little difference between the oral (ig) and inhalation groups in serum enzyme levels. The only index of liver injury showing a more pronounced effect in the ig animals was microsomal P-450, which was lower in the oral than in the inhalation group at the high dosage level. It should be noted that the administered inhaled dose was utilized (given orally) here. Experiments are currently in progress to determine the actual absorbed dose during CCl_4 inhalation. This dose will be administered to other rats by ig infusion over the same time-frame, and CCl_4 PK and hepatoxicity assessed, in order to more adequately ascertain the influence

of route of exposure on CCl_4 PK and target organ toxicity. Results of experiments conducted to date were presented at a conference on risk assessment, which was cosponsored by the EPA and ILSI in 1990, and published the same year (Bruckner *et al.*, 1990a).

B. Characterization of Presystemic Elimination of Ingested VOCs

One of the major objectives of the project is to determine the relative significance of first pass elimination of ingested halocarbons by the liver and lungs. Although there is very limited information about the roles that "first pass" metabolism/elimination by the liver and lungs play, it seems likely that substantial proportions of some VOCs absorbed from the GI tract will be metabolically degraded and/or exhaled before reaching the systemic circulation and extrahepatic target organs. Relatively low doses, as are encountered environmentally, should be most significantly affected.

In light of the foregoing, experiments were undertaken to elucidate the influence of oral dosage regimen on the systemic uptake, disposition and elimination of a series of halocarbons. Although all of the halocarbons selected for study were volatile, two were extensively metabolized (i.e. trichloroethylene (TCE) and 1,1-dichloroethylene (1,1-DCE)) and two were poorly metabolized (1,1,1-trichloroethane (TRI) and perchloroethylene (PER)). Although patterns of water consumption vary in human populations, constant gastric infusion were selected to approximate frequent, repetitive ingestion of water. Equivalent doses of each halocarbon were given to unanesthetized, male S.D. rats as an aqueous Emulphor® emulsion, either orally as a single bolus or infused through a surgically-implanted gastric cannula for 2 hours. The animals were surgically prepared the day before by implantation of a cannula into their right carotid artery, so that serial blood samples could subsequently be taken from unanesthetized, freely-moving animals. Periodic blood samples of 25-50 µl were taken via the carotid cannula and analyzed for halocarbon content by a headspace technique in a gas chromatograph equipped with an electron capture detector. Blood concentration versus time profiles were thereby obtained and a variety of pharmacokinetic (PK) parameters determined.

Data analysis revealed marked PK differences between the oral bolus and intragastric (ig) infusion groups. Significant reductions in area under the blood concentration versus time curve (AUC) and peak blood concentration (C_{max}), and increased (blood) elimination half-life ($t^{1/2}$) were observed for each halocarbon, when it was given by ig infusion (versus bolus dosing). Blood concentrations of the 2 well metabolized halocarbons studied, TCE and 1,1-DCE, were so low that they were hardly detectable at low dosage levels in the ig animals. These findings may be very important implications in toxicity and cancer risk assessment, in that the liver and lungs may be able to remove virtually all of the trace amounts of VOCs that are normally found in drinking water and foods.

The aforementioned findings will be included in 3 publications, one each for 1,1-DCE, TCE, and TRI. The 1,1-DCE paper is currently in draft form and will be submitted for publication this coming year. The findings for the other VOCs were reported at 1990 SETAC meeting (Bruckner *et al.*, 1990b).

Analysis of blood concentration versus time profiles, in groups of animals given the VOCs by different routes of administration, is the most expeditious way to directly assess first-pass hepatic and pulmonary uptake of VOCs. To discern the relative contributions of the GI tract, liver and lung to first pass elimination, a series of equivalent doses of selected chemicals will be administered via the portal vein (through a surgically implanted cannula), intravenously (iv), intraarterially (ia) and orally (po). Determination of the area under the blood concentration time curves (AUCs) for each administration site will allow the fractional contribution of each organ to the first pass effects to be assessed.

After the completion of these experiments, there will be data from the following exposure scenarios:

Exposure route	Administration site	Sampling site
iv	jugular vein	carotid artery
po	stomach via gastric tube	carotid artery
portal vein	portal vein	carotid artery
ia	carotid artery	femoral artery

Bioavailability (F) represents the fraction of the dose that reaches the systemic circulation. It can be expressed as: $F = f_g f_h f_l$ where f_g = the fraction of dose escaping GI metabolism or elimination, f_h = the fraction of dose escaping hepatic metabolism or elimination, and f_l = the fraction of dose escaping lung metabolism or elimination. These fractional components of F can be assessed as follows: $f_g = (AUC)_{po}/(AUC)_{pv}$, $f_h = (AUC)_{pv}/(AUC)_{iv}$, and $f_l = (AUC)_{iv}/(AUC)_{ia}$ where $(AUC)_{po}$ = the area under the blood VOC concentration-time curve from time zero to infinity following oral administration, $(AUC)_{pv}$ = the area under the blood VOC concentration-time curve from time zero to infinity following portal vein injection, $(AUC)_{iv}$ = the area under the blood VOC concentration-time curve from time zero to infinity following jugular vein injection, and $(AUC)_{ia}$ = the area under the blood VOC concentration-time curve from time zero to infinity following carotid artery injection. The product of f_g, f_h and f_l is equal to $(AUC)_{po}/(AUC)_{ia}$ or F. By evaluating F_g, f_h and f_l therefore F as a function of dose, the extent and source of saturable presystemic elimination of VOCs can be ascertained.

We have initiated an investigation of the first pass, or presystemic elimination of TCE. Our aims are to determine the relative contribution of the liver and lungs, as well to delineate the dose-dependency of first pass uptake. Cannulas are surgically implanted into male S.D. rats 24-48 hours before TCE dosing. TCE (0.6-14 mg/kg bw) in a 5% aqueous Emulphor® emulsion is injected over 30 sec into the hepatic portal vein (PV), jugular vein or carotid artery. Serial blood samples of 5-200 µl are collected for up to 10 hours from the carotid or femoral artery of the unanesthetized rats and analyzed for TCE content by headspace gas chromatography. Results to date indicate non-linearity in TCE elimination at PV doses higher than 7 mg/kg, as manifest by diminished clearance values. Total presystemic elimination is inversely related to dose, ranging from >50% to <10%. The liver appears to be responsible for about 3/4, the lungs 1/4

of presystemic elimination of TCE. These findings indicate that a substantial fraction of trace amounts of VOCs ingested in environmental media may not enter the systemic circulation nor reach extrahepatic target organs.

C. Investigation of the Role of the Lymphatics in GI Absorption of VOCs and the Influence of Corn Oil on GI Absorption Pathways

Although the lymphatics represent a major pathway for absorption of long-chain dietary fatty acids, there is very little information about the role of the lymphatics in GI absorption of VOCs and other hydrocarbons. It is possible that ingestion of VOCs in a digestible oil may result in their incorporation into chylomicrons and entry into the lacteals rather than the blood. If VOCs do enter the lymphatic vessels rather than the portal blood, the chemicals will not be subject to first pass metabolism and elimination. Also, certain chemicals may exert immunotoxic effects within the lymphatic system.

An investigation was conducted to: (a) determine whether an orally administered halocarbon (i.e. CCl_4) was absorbed via the lymphatics; (b) evaluate the relative importance of the lymphatics versus the portal (blood) system in GI absorption; (c) examine the influence of oil dosing vehicles on the absorption pathways. Male S.D. rats were surgically prepared with indwelling thoracic duct and jugular vein cannulas. The thoracic duct is the major lymphatic vessel in the body. The distal ends of the two cannulas were exteriorized through the same incision and connected, in order to reestablish the return of the lymph to the venous circulation during the animal's 24-hour recovery period. Each cannulated rat was administered CCl_4 for 99+% purity as a single oral bolus of 25 mg/kg: (a) in corn oil; (b) as an 0.5% aqueous Emulphor® emulsion; and (c) in water. Lymph was collected under toluene continuously for up to 10 hours after dosing. Serial venous blood samples were taken during this time period from another group of animals. The CCl_4 content of the blood and lymph samples was measured by gas chromatography headspace analysis.

Data from the foregoing absorption pathways study revealed that orally administered CCl_4 was absorbed concurrently into the lymphatic and the venous circulatory systems. The proportion of chemical absorbed via the lymphatics was quite small, relative to that taken up into the portal venous blood. The dosage vehicle/diluent had a pronounced effect on the absorption pathways. Approximately 0.75% of the 25 mg/kg dose was estimated to be absorbed via the lymphatics, when it was given in corn oil, versus only 0.043% and 0.058% for the emulsion and water groups, respectively. The CCl_4 concentration was 10- to 30-fold higher in the lymph of the corn oil animals than in that of the emulsion and water animals. Moreover, the CCl_4 concentration in the corn oil group was 10- to 100-fold higher in lymph than in blood during the 10-hour monitoring period. This phenomenon may have important implications in the interpretation of immunology and cancer bioassays, in which VOCs and other organic chemicals have been routinely administered to rodents in oil vehicles. The relatively high chemical concentrations in the lymph may suppress immune function, possibly resulting in increased expression of chemically-induced cell mutations and cancers.

The lymph and blood CCl_4 concentration versus time profiles of rats given the chemical in corn oil were quite different, qualitatively as well as

quantitatively, from profiles in the aqueous vehicle groups. CCl_4 levels increased very quickly following dosing in both venous blood and lymph in the water and emulsion groups, indicating rapid absorption of the halocarbon from the GI tract. Concentrations of CCl_4 diminished more slowly in the lymph than in the blood during the 10-hour post-exposure period in both aqueous vehicle groups. In contrast, CCl_4 levels in lymph and blood exhibited prolonged elevation, with multiple secondary peaks. This "plateau phenomenon" can be ascribed to the marked delay and prolongation of CCl_4 absorption by corn oil seen in a previous study (Kim *et al.*, 1990b). Despite the prolonged, relatively high concentrations of CCl_4 in lymph, the portal (venous) blood accounted for the majority of CCl_4 absorption. This is attributed to the markedly greater flow rate in the portal circulation. Results of this investigation were presented at the 1990 Society of Toxicology meeting (Kim *et al.*, 1990d), and will be incorporated into a paper to be submitted for publication this coming year.

D. <u>Characterization of the Influence of Oral Dosage Vehicles on the Absorption, Pharmacokinetics and Toxicity of VOCs</u>

A large number of VOCs are of major health concern as drinking water contaminants. It has been necessary in most oral toxicity studies to give the VOCs in oil dosage vehicles, due to their limited water solubility. This routine use of <u>oil</u>-based vehicles may <u>introduce confounding factors</u>, which could substantially affect the <u>relevancy</u> of study results to risk assessment of VOCs in drinking water.

The objective of the first series of studies we undertook was to assess the influence of dosing vehicles on the pharmacokinetics (PK) and the acute hepatotoxicity of CCl_4. In one experiment, fasted male S.D. rats were given a series of doses of CCl_4 by gavage: in corn oil; as an aqueous Emulphor® emulsion; as the undiluted chemical; and in water. Blood and liver samples were taken 24 hours post dosing for measurement of serum and hepatic microsomal enzymes and for histopathological examination. Acute hepatotoxicity, as reflected by these parameters, was less pronounced at each dosage level in rats given CCl_4 in corn oil than in the other vehicles. In contrast, the aqueous emulsion did not substantially alter the toxicity of CCl_4 from that of undiluted CCl_4 or CCl_4 ingested in water.

In the companion experiment, rats received a single dose (25 mg/kg) of CCl_4 by gavage: in corn oil; as an aqueous Emulphor® emulsion; as the undiluted chemical; and in water. The 25 mg/kg dose was given to a second group of rats iv through an indwelling jugular cannula. Serial blood samples were taken from both groups via a surgically-implanted carotid artery cannula and analyzed for CCl_4 content by headspace gas chromatography, in order to obtain blood concentration versus time profiles. CCl_4 was absorbed very rapidly from the GI tract, as peak blood concentrations were reached within 3 to 6 minutes after dosing in the aqueous emulsion and water groups. These peak levels were much higher than in the corn oil group. Corn oil markedly delayed the absorption of CCl_4 from the GI tract and produced secondary peaks in the blood profiles. Elimination from the blood stream of the iv group followed a triexponential pattern. There was a high degree of correlation of both C_{max} and AUC_0^{120} with the magnitude of hepatotoxicity. <u>CCl_4 was less toxic in corn oil</u> due to <u>delay</u> and prolongation of CCl_4 absorption, resulting in a <u>marked decrease</u> in CCl_4

concentration in the arterial blood and likely in the liver. These findings demonstrate that corn oil has sufficient effect on the PK and acute hepatotoxicity of CCl_4 to warrant reappraisal of the use of oil dosage v .icles in toxicity studies of VOCs. The use of aqueous Emulphor● emulsions, however, appears appropriate in studies of VOC contaminants of drinking water, in that the emulsion did not substantially alter the PK or toxicity from that of CCl_4 ingested in water. These findings were published in two papers (Kim *et al.*, 1990a & b).

Effort was also devoted to PK analysis and computer modeling of the GI absorption of VOCs from oil diluents. Ramsey and Andersen (TAP 73:159-175, (1974), in a "benchmark" paper on physiologically-based pharmacokinetic (PBPK) modeling of VOCS, could not model the GI uptake and disposition for styrene from vegetable oil. They concluded that more complex modeling efforts would be required to simulate and predict the effect of oils on the systemic absorption of VOCs. A systems analysis approach was thus used to analyze the data from the CCl_4 PK studies described in the preceding paragraph. The systems analysis approach makes use of the convolution-deconvolution relationship to describe the output and input into a system. Unlike classical compartmental modeling approaches that typically assume first- or zero-order absorption processes, the emphasis in systems analysis is on obtaining a function that characterizes the system outputs (i.e., blood concentration versus time data). Estimation of the cumulative percent of CCl_4 absorbed over time by deconvolution indicated that corn oil will result in prolonged, erratic absorption of CCl_4. This was indeed the case, as evidenced in the blood profiles of animals dosed with CCl_4 in corn oil by a long T_{max} and pronounced intersubject variability. The systems analysis modeling approach was presented at an EPA- and ILSI-sponsored risk assessment conference in 1990, and published that same year (Gillespie *et al.*, 1990). Subsequent work was done to evaluate the usefulness of linear systems analysis as an adjunct to PBPK modeling. A PBPK model, employing an absorption input rate function provided by systems analysis, accurately predicted blood CCl_4 concentration-time date for both aqueous and oil oral dosage vehicles. These findings are being incorporated into a manuscript which will be submitted for publication next year.

Studies by several groups of investigators have shown that the oral subchronic toxicity and carcinogenicity of VOCs can be significantly altered by the gavage vehicle. Chloroform ($CHCl_3$) and a number of other halocarbons have been found to produce a very high incidence of hepatocellular carcinoma, when given to B6C3F1 mice in corn oil by gavage. Jorgenson *et al.* (FAAT 5:760-769, 1985), however, saw no evidence of tumorigenesis when these mice were given the same doses of $CHCl_3$ in drinking water. Similarly, Klaunig *et al.*, (Envir. Health Perspec. 69:89-95, 1986) found that $CHCl_3$, 1,1-DCE and 1,2-dichloroethane were not carcinogenic when given to mice in their drinking water, although each VOC was reported to be a hepatocarcinogen when administered by gavage in corn oil. CCl_4 and $CHCl_3$ have been reported by other researchers to be more hepatotoxic when given to mice for 90 days in corn oil, than when given in an aqueous suspension. We conducted 2 subchronic studies of the hepatotoxicity of CCl_4 given orally to rats in corn oil versus aqueous vehicles. In the first, a battery of indices consistently indicated CCl_4 to be somewhat more hepatotoxic after 2 and 4 weeks of dosing when given in corn oil. In the second study, CCl_4 given in corn oil was slightly more hepatotoxic at 4 and 8 weeks, but liver injury was of similar

magnitude after 13 weeks in the corn oil and aqueous vehicle groups. The results of one of these studies were presented in detail at the 1990 Society of Toxicology meeting (Koporec *et al.*, 1990). Two manuscripts describing the work will be submitted for publication in 1991. It would appear that the aforementioned dissimilarities in hepatotoxic and carcinogenic potency of halocarbons in different studies may be attributable to differences in both dosage vehicle and dosage regimen (i.e., bolus versus divided doses).

E. Investigation of Potential Mechanisms by Which Corn Oil Alters the Pharmacokinetics and Toxicity of Orally Administered VOCs

The final objective of this project is to examine possible mechanisms by which corn oil affects the pharmacokinetic (PK) and toxicity of ingested VOCs. Studies have already been discussed which clarify some of the mechanisms of alteration of the PK and toxicity of a model halocarbon, CCl_4. Corn oil delayed and prolonged the GI absorption of CCl_4, resulting in a significant decrease in its acute hepatotoxicity, but some increase in toxicity during the initial weeks of subchronic CCl_4 exposure. Elevated lipid intake from corn oil caused increased deposition of triglycerides in the liver, which in turn resulted in increased deposition of CCl_4. Although it has been suggested that high dietary corn oil may enhance lipoperoxidation in liver microsomes and mitochondria, as well as increase the superoxide and peroxide content of the liver, our first subchronic study showed that corn oil did not cause an increase in hepatic microsomal lipid peroxidation in CCl_4-treated or vehicle control animals.

An investigation in our laboratories revealed that dietary polyunsaturated fatty acids are needed for optimal activity and induction of hepatic microsomal P-450 in rats. Male S.D. rats were starved for 36 hours, and then refed a fat-free (FF) diet or a diety containing 20% corn oil (COD) for 4 days. Some rats were dosed with phenobarbital (PB) daily for 3 days during this period. PB-treated FF animals had only 21% more P-450 than FF controls, whereas rats fed the 20% COD had 59% more P-450, and the PB-treated COD rats had 181% more P-450 than the FF controls. Five P-450 isozymes separated by SDS-PAGE were quantified using a gel scanner. Analysis of the gels showed 32, 59, and 124% more P-450 (total isozymes) in FF PB, COD, and COD PB groups, respectively, than in the FF groups. These findings suggest that dietary fat deprivation reduces the total amount of cytochrome P-450 hemoprotein and its inductibility by PB through decreased P-450 hemoprotein synthesis. The limiting factor(s) restricting synthesis of new cytochrome P-450 hemoprotein in rats devoid of fat may be an inability to respond to the inducer (PB), or the deficit of fatty acids needed for synthesis of the phospholipid matrix of the microsomal membrane necessary for support and proper juxtapositioning of these enzymes. These findings were incorporated into a manuscript which was recently published (Kim *et al.*, 1990c).

A study was initiated to evaluate the effect of dietary fat deprivation on the acute toxicity of CCl_4. Male S.D. rats were starved for 36 hours and then refed a FF diet or a 20% corn oil diet (COD) for 4 days. Some animals received phenobarbital (PB) sodium (80 mg/kg, ip daily) for 3 days. One half of the PB-treated and control rats were given 250 mg CCl_4/kg bw. Blood and liver samples were taken 24 hours after dosing for measurement of serum and microsomal enzymes and histopathological examination. Hepatic microsomal proteins were separated by SDS-PAGE and P-450 isozymes quantitated with a gel scanner. Significantly

greater damage to hepatocytes was evident in rats fed the COD by elevated serum enzyme levels and histologic examination. Whereas CCl_4 produced no destruction of P-450 in FF rats, it decreased P-450 levels in COD rats by nearly 50%. CCl_4 reduced P-450 160% in COD-PB rats. CCl_4 significantly reduced P-450 isozymes in COD rats, while having no effect on isozyme levels of FF rats. It was possible to gain insight into the role of different P-450 isozymes in the bioactivation of CCl_4 (to cytotoxic metabolites), by correlation of the differential effects of the FF diet, COD and PB on individal P-450 isozymes and the extent of CCl_4-induced liver injury. Two isozymes (i.e., 51.2 and 53 kd components), in particular, were induced by PB and appeared to be active in potentiation of CCl_4 hepatotoxicity by PB. These 2 constitutive forms of P-450, known collectively as the PB-B form of the enzyme, seemed to be primarily <u>responsible for metabolic activation of CCl_4</u>. These findings were reported at the 1990 Society of Toxicology meeting (Choi *et al.*, 1990) and have been included in a manuscript to be submitted for publication next year.

One of the stated aims of the project is to test the hypothesis that corn oil enhances the elimination (and toxicity) of VOCs by induction of liver mixed-function oxidase (MFO) activity. Although we have demonstrated that dietary lipids are required for synthesis of P-450 hemoproteins and metabolism of xenobiotics, it is still not clear whether a substantial increase in lipid intake will produce an increase in P-450 levels and metabolic capacity over what is usually present in animals consuming a normal diet. This question has <u>important</u> implications. VOCs are typically given to animals by corn oil gavage in toxicology and cancer studies. If the high doses of corn oil induce P-450 and MFO activities, the metabolism and toxicity or carcinogenicity of the test chemical may be significantly altered.

We undertook a study of the influence of corn oil (CO) ingestion on hepatic microsomal cytochrome P-450 and its isozymes. The objectives of the study were to determine whether short- and long-term, high-level corn oil ingestion caused: (a) a change in total P-450 levels; (b) an alteration in the profile of major P-450 isozymes; and (c) a difference in the metabolism of selected chemicals. Male S.D. rats were given 5 ml CO/kg bw daily by gavage for up to 13 weeks. Groups of 5 rats were sacrificed at 1 day and at 1, 2, 4, 8, and 13 weeks. The liver was removed and total microsomal cytochrome P-450 measured spectrophotometrically. Hepatic microsomal proteins were separated by SDS-PAGE and P-450 isozymes quantitated with a gel scanner. Benzo(a)pyrene hydroxylase, ethymorphine demethylase (EMD) and N-nitrosodimethylamine demethylase (NDMD) activities were measured by standard techniques. There were no significant differences between CO and control groups in total P-450 or in constitutive P-450 isozymes during the 13-weeks. MFO activities occasionally appeared somewhat lower in the CO animals during the period, but only at 13 weeks were the decreases from controls in EMD and NDMD activities statistically significant. Thus, <u>excessive CO intake</u> does <u>not</u> appear to induce, nor to substantially alter hepatic P-450s or MFO activity. These findings were recently presented at the summer 1990 ASPET (Pharmacology) meeting (Kim and Bruckner, 1990), and will be submitted for publication in 1991.

IV. PUBLISHED ABSTRACTS AND JOURNAL ARTICLES

Bruckner, J.V., Kim, H.J., Muralidhara, S., and Gallo, J.M. Influence of Route and Pattern of Exposure on the Pharmacokinetics and Hepatotoxicity of Carbon Tetrachloride. In: Principles of Route-To-Route Extrapolation for Risk Assessment, Gerrity TR and Henry CJ, eds., pp. 271-284, Elsevier, New York (1990a).

Bruckner, J.V., Dallas, C.E., Muralidhara, S., Srivatsan, V., Manning, R.O., and Gallo, J.M. Presystemic Elimination of Volatile Organic Compounds (VOCs) Ingested in Water. Society of Environmental Toxicology and Chemistry 11: 240 (1990b).

Choi, C.W., Kim, H.J., Jun, H.W., Voss, K.A., Bruckner, J.V., and Wade, A.E. Alteration of carbon tetrachloride (CCl_4) hepatotoxicity by dietary fat. Toxicologist 10: 198 (1990).

Gillespie, W.R. Cheung, L.L., Kim, H.J., Bruckner, J.V., and Gallo, J.M. Application of Systemic Analysis to Toxicology: Characterization of Carbon Tetrachloride Oral Absorption Kinetics. In: Principles of Route-To-Route Extrapolation for Risk Assessment, Gerrity TR and Henry CJ, eds., pp. 285-295, Elsevier, New York (1990).

Kim, H.J., Odend'hal, S., and Bruckner, J.V. Effect of oral dosing vehicles on acute hepatotoxicity of carbon tetrachloride in rats. Toxicol. Appl. Pharmacol. 102: 34-49 (1990a).

Kim, H.J., Gallo, J.M., Dallas, C.E., and Bruckner, J.V. Effect of oral dosing vehicles on the pharmacokinetics of orally administered carbon tetrachloride in rats. Toxicol. Appl. Pharmacol. 102: 50-60 (1990b).

Kim, H.J., Choi, E.S., and Wade, A.E. Effect of dietary fat on the induction of hepatic microsomal P-450 isozymes by phenobarbital. Biochem. Pharmacol. 39: 1423-1430 (1990c).

Kim, H.J., Muralidhara, S., Choi, C.W., and Bruckner, J.V. Role of the lymphatics in absorption of carbon tetrachloride (CCl_4) given orally in different dosing vehicles. Toxicologist 10: 52 (1990d).

Kim, C. and Bruckner, J.V. Effect of increased corn oil ingestion on hepatic microsomal oytochrome P-450 and mixed-function oxidase (MFO) activity. Pharmacologist 32: 174 (1990).

Koporec, K.P., Kim, H.J., MacKenzie, W.F., and Bruckner, J.V. Evaluation of oral dosing vehicle effects on the subchronic hepatotoxicity of carbon tetrachloride (CCl_4) in the rat. Toxicologist 10: 52 (1990).

Manning, R.O., Brown, K.H., Srivatsan, V., Gallo, J.M., and Bruckner, J.V. Pharmacokinetics of trans-1,2-dichloroethylene (DCE) and 1,1-dichloroethane (DCA) in rats. Toxicologist 10: 235 (1990).

V. PROFESSIONAL ACTIVITIES RELATED TO PROJECT TOPICS

Dr. Bruckner presented a seminar on factors which influence halogenated hydrocarbon toxicity at the U.S. EPA Health Effects Research Laboratory in Research Triangle Park, NC, February, 1990.

Drs. Bruckner, Dallas, Hyo, and Manning and Mr. Muralidhara attended and presented research papers at the annual meeting of the Society of Toxicology in Miami, FL, February, 1990.

Drs. Bruckner and Gallo presented invited papers at the Principles of Route-to-Route Extrapolation for Risk Assessment Workshop, sponsored by the U.S. EPA and ILSI, Hilton Head, SC, March, 1990.

Dr. Gallo attended and made a presentation on physiologically-based pharmacokinetic modeling at the 23rd annual Higuchi Research Conference in Ozark, MO, March, 1990.

Dr. Bruckner served as a reviewer for Toxicological Profiles for Trichloroethylene, 1,1-Dichloroethane and 1,1,2-Trichloroethane for the U.S. Agency for Toxic Substances and Disease Registry.

Drs. Bruckner and Dallas gave invited research presentations at the 11th annual meeting of the Society of Environmental Toxicology and Chemistry in Arlington,, VA, November, 1990.

Dr. Bruckner was a member of the Committee on Pesticides in the Diets of Infants and children, for the Board on Agriculture and the Board on Environmental Studies and Toxicology, National Academy of Sciences.

Dr. Bruckner served on a working group on chemical contaminants in reclaimed water for the space station, National Academy of Sciences and the National Aeronautics and Space Administration.

Dr. Bruckner served as a consultant to Shell, Occidental, Exxon, and other companies having problems with groundwater contamination by volatile organic chemicals.

Drs. Bruckner, Dallas, Gallo, and Manning served as editorial board members and reviewers for scientific journals, in processing research manuscripts pertaining to the toxicity and pharmacokinetics of volatile organic chemicals and other compounds.

VI. ADVANCED DEGREE AWARDED

Kevin P. Koporec, M.S., June, 1990. Effect of Oral Dosing Vehicles on the Subchronic Hepatotoxicity of Carbon Tetrachloride (CCl_4) in the Rat.